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(54) Title: **METHOD FOR THE DIAGNOSIS AND FOLLOW UP OF SCHIZOPHRENIA AND OTHER MENTAL AND NEURODEGENERATIVE DISORDERS**

(57) Abstract: A method for the diagnosis and follow up of a mental disorder or of a neurodegenerative disorder in an individual, comprises: (i) measuring mRNA of D<sub>3</sub> dopamine receptor and/or of  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$  AChR) and of a control gene in peripheral blood lymphocytes (PBLs) of said individual and of at least one healthy control individual; (ii) calculating the ratio between the D<sub>3</sub> dopamine receptor mRNA and the control gene mRNA and/or the ratio between  $\alpha 7$  AChR mRNA and the control gene mRNA for each individual; and (iii) evaluating the ratio between the ratios obtained in (ii) for the tested individual and for the at least one healthy control individual, wherein an increase in the D<sub>3</sub> dopamine receptor mRNA and/or a decrease in the  $\alpha 7$  AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood of having said mental disorder or neurodegenerative disorder, wherein said increase in the D<sub>3</sub> dopamine receptor mRNA and/or decrease in the  $\alpha 7$  AChR mRNA in the tested individual is correlated to said mental disorder or neurodegenerative disorder. When the mental disorder is schizophrenia, an increase of above 1.6 fold in the D<sub>3</sub> dopamine receptor mRNA and/or a decrease of more than 20% in the  $\alpha 7$  AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood of having schizophrenia.

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## METHOD FOR THE DIAGNOSIS AND FOLLOW UP OF SCHIZOPHRENIA AND OTHER MENTAL AND NEURODEGENERATIVE DISORDERS

### 5 Field of the Invention

The present invention relates to methods for the diagnosis and follow-up of schizophrenia and other mental and neurodegenerative disorders, and kits for use in said methods.

### 10 Background of the Invention

Schizophrenia is a neuropsychiatric disorder afflicting about one percent of the population. It is characterized by delusions, hallucinations, disorders in organizing thoughts logically, and emotional withdrawal. There is a well-known tendency for schizophrenia to run in families.

15 Although the exact pathogenesis of schizophrenia is still not known precisely, a common belief is that excessive activity at dopaminergic synapses in the brain plays a prominent role. To date, a definitive diagnosis of schizophrenia requires a 6-month duration of symptomatology, and relies on heterogeneous symptoms. Because there is neither an effective biological marker for identifying schizophrenia (Willner, 1997;  
20 Hietala and Syvalahti, 1996), nor an accurate and rapid diagnosis to ensure more optimal management at an early stage in the illness, there remains a vital need for a convenient assay for diagnosis and follow-up of schizophrenia.

Most of the drugs used to treat schizophrenia act to control the symptoms by neuroreceptor antagonism. Moreover, the dopaminergic basis of schizophrenia is  
25 strongly supported by the close correlation between clinical efficacy of antipsychotic medications and their potency to antagonize the binding of dopamine to its receptors (Creese et al., 1976).

Dopamine receptors are divided into two subclasses D1 and D2. The D1 subclass contains the D<sub>1</sub> and D<sub>5</sub> receptor subtypes, and the D2 subclass contains the D<sub>2</sub>,  
30 D<sub>3</sub> and D<sub>4</sub> subtypes (Levant, 1997). The dopamine hypothesis of schizophrenia relates specifically to the D2 subclass. Notably, most drugs effective in treating schizophrenia exhibit D2 receptor antagonistic activity, and administration of a selective D1-like antagonist has been reported to result in the worsening of symptoms (Karlsson et al.,

1995). Among the receptors in the D2 subclass (D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>), the D<sub>3</sub> receptor is located principally in an area of the brain that could be very relevant to schizophrenia, the nucleus accumbens (Willner, 1997). Studies with positron-emission tomography and postmortem brain tissue have indicated increased levels of D2-like dopamine receptors in schizophrenics when compared with nonschizophrenic patients (Seeman and Niznik, 1990). Thus, the level of dopamine receptor could be employed as a marker for schizophrenia if it could be analyzed on an available tissue, preferably a peripheral one.

High affinity binding of dopaminergic ligands, as well as the presence of mRNA of several dopamine receptor subtypes (D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub>) in human peripheral blood lymphocytes (PBLs) have been reported in recent years (Ricci et al., 1997, Takahashi et al., 1992). It should be noted, however, that neither D<sub>2</sub> nor D<sub>1</sub> dopamine receptor subtypes, which are the most abundant receptors in the brain and belong to the D2 and the D1 subclasses, respectively, have been detected in lymphocytes. Although the significance of dopamine receptors, as well as of other neurotransmitter receptors, in lymphocytes is still not clear, it has been suggested that they may reflect corresponding brain receptors. Several studies have demonstrated the increased binding of dopamine antagonists in lymphocytes of schizophrenic patients as compared with healthy individuals (Bondy et al., 1984; Bondy et al., 1985). In addition, a previous study carried out in the laboratory of the present inventors has demonstrated that spiperone (a D2 antagonist) binding in peripheral blood lymphocytes is higher in neuroleptic responders as compared with treatment-resistant schizophrenic patients (Grodzicki et al., 1990). However, the observed differences in binding studies were rather low and often not significant. The discrepancies obtained could have resulted from the crossreactivity of radioligands with different subtypes of the receptor and with other receptors (e.g. serotonergic), and from scattered levels of binding sites. Therefore, such binding assays in lymphocytes may not be suitable for a reliable assay for schizophrenia.

Such a correlation between the status of receptors in the brain and in PBLs has also been demonstrated in Alzheimer's disease, where muscarinic receptors are reduced in both brains and lymphocytes (Ferrero et al., 1991). A previous study by Nagai et al. (1996) demonstrated that patients with Parkinson's disease exhibit reduced levels of D<sub>3</sub> receptor mRNA in PBLs, as compared with healthy individuals. These latter findings provide another example of a disease that is associated with an insult in the central

nervous system that is reflected in PBLs. This reduction has also been detected in medicated and non-medicated patients.

Central cholinergic systems were also shown to control basic functions of the brain. Acetylcholine mediates synaptic transmission in the vertebrate central nervous system through the activation of two major receptor subtypes, the muscarinic and  
5 nicotinic acetylcholine receptors (AChRs). The muscarinic receptors are G-coupled receptors, and the nicotinic receptors are ligand-gated ionic channels. Nicotinic AChRs are composed of five subunits organized around a central ion channel. Neuronal nicotinic AChRs are usually built as heteropentamers, composed of  $\alpha$ ( $\alpha$ 2- $\alpha$ 9), and  $\beta$ ( $\beta$ 2- $\beta$ 4)  
10 subunits.  $\alpha$ 7,  $\alpha$ 8, and  $\alpha$ 9 can function as homomeric AChRs and are of special interest because they bind the curarimetric neurotoxin,  $\alpha$ -bungarotoxin. ( $\alpha$ -BTX $\beta$ ). These receptors are characterized by a rapid rate of desensitization, and a high level of selectivity to calcium.

Several recent studies have suggested that nicotinic  $\alpha$ 7 AChR may be associated  
15 with some aspects of schizophrenia (Guan et al., 1999). Nicotine administration normalizes two psychophysiological deficits, typical for schizophrenia: disordered eye movements, and the P50 auditory evoked potential gating deficit (Olincy et al., 1998). The genes responsible for these two deficits are linked genetically to the chromosomal locus (15q14) of the  $\alpha$ 7-nicotinic receptor gene (Leonard et al., 2000).  $\alpha$ 7 AChR has  
20 been found to be expressed in the mammalian brain, especially throughout the hippocampus (Hellstrom-Lindahl et al., 1999), a brain region associated with schizophrenia.

Interestingly, the vast majority of schizophrenic patients are smoking. They appear to extract more nicotine than normal smokers, possibly due to different inhalation  
25 patterns (Olincy et al., 1997). This fact raised the possibility that nicotine might influence the levels of  $\alpha$ 7 receptor. However, searching for receptor differences between smokers and nonsmokers in the general population did not reveal any significant differences (Stassen et al., 2000).

Association between the  $\alpha$ 7 nicotinic receptor levels and Alzheimer's disease has  
30 also been investigated. Decrease in the expression of  $\alpha$ 7 AChR was observed in post mortem tissue from Alzheimer's disease patients, exhibiting a reduction of 36% in the hippocampus (Guan et al., 2000). Burghaus et. al. (2000) reported a decrease in protein amount of  $\alpha$ 7 AChR in Alzheimer's disease cortices. Wang et. al. (2000) described an

interaction of  $\alpha 7$  AChR and  $\beta$ -amyloid (1-42) as a mechanism involved in the pathophysiology of Alzheimer's disease. There have been some other conflicting reports demonstrating higher levels of the  $\alpha 7$  AChR mRNA in the hippocampus (Hellstrom-Lindahl et al., 1999) as well as in lymphocytes (Hellstrom-Lindahl et al., 1997) of  
5 Alzheimer's disease patients, compared to healthy controls.

Freedman et al. (2000) reported that interneurons in the hippocampus and in other forebrain structures are decreased in number and function in subjects with schizophrenia. Decreased  $\alpha 7$ -nicotinic receptor immunoreactivity was found in the frontal cortex and in the nucleus reticularis thalami of schizophrenic patients (Freedman  
10 et al., 2000). Court et. al. (1999) described a reduction in the  $\alpha$ -BTX binding, and no significant alterations in the nicotine binding in post mortem brains of schizophrenic patients. A significant decrease in the level of  $\alpha 7$  AChR was also observed by Guan et. al. (1999) in the frontal cortex of schizophrenics when compared with controls, suggesting that  $\alpha 7$  AChR may be involved in inhibitory neuronal pathways engaged in  
15 this disorder.

#### **Summary of the Invention**

According to the present invention, we measured the mRNA levels of dopamine receptors and of  $\alpha 7$  nicotinic acetylcholine receptor (AChR) in peripheral blood  
20 lymphocytes (PBLs) of schizophrenics and healthy individuals in order to find out if they can serve as peripheral markers for this disorder. Since the inhibitory D2 subclass of dopamine receptors is considered to be associated with neuropsychiatric disorders rather than the D1 subclass, we have focused only on the D<sub>3</sub> and D<sub>4</sub> subtypes, both belonging to the D2 subclass. We have then found a correlation between D<sub>3</sub> dopamine receptor on  
25 lymphocytes and schizophrenia, showing a significant elevation of above about 1.6, particularly 2-4, folds in mRNA level of D<sub>3</sub> but not of D<sub>4</sub>, in the schizophrenic patients. In addition, a significant decrease (> 20%, particularly 20-98%) of the  $\alpha 7$  AChR mRNA levels in PBLs of schizophrenic patients was observed. The changes in the mRNA level of the D<sub>3</sub> dopamine receptor and of the  $\alpha 7$  AChR in schizophrenic patients are not  
30 affected by different drug treatments. Moreover, non-medicated patients exhibit the same pattern, indicating that these changes are not a result of the medical treatment.

The present invention thus relates to the evaluation of the mRNA levels of D<sub>3</sub> dopamine receptor and/or of  $\alpha 7$  AChR in PBLs of an individual as reliable peripheral markers for the identification and follow-up of schizophrenia, of other mental disorders, and of neurodegenerative disorders.

5 In one aspect, the invention relates to a method for the diagnosis and follow-up of a mental disorder or of a neurodegenerative disorder in an individual, comprising:

- (i) measuring mRNA of D<sub>3</sub> dopamine receptor and/or of  $\alpha 7$  AChR, and of a control gene in peripheral blood lymphocytes (PBLs) of said individual and of at least one healthy control individual;
- 10 (ii) calculating the ratio between the D<sub>3</sub> dopamine receptor mRNA and the control gene mRNA, and/or the ratio between  $\alpha 7$  AChR mRNA and the control gene mRNA for each individual; and
- (iii) evaluating the ratio between the ratios obtained in (ii) for the tested individual and for the at least one healthy control individual, wherein an increase in the ratio of the
- 15 D<sub>3</sub> dopamine receptor mRNA and/or a decrease in the ratio of the  $\alpha 7$  AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood of having said mental disorder or neurodegenerative disorder, wherein said increase in the D<sub>3</sub> dopamine receptor mRNA and/or decrease in the  $\alpha 7$  AChR mRNA in the tested individual is correlated to said mental disorder or
- 20 neurodegenerative disorder.

The mental disorder may be, for example, schizophrenia, maniac depression, Tourette syndrome or a similar disorder, and the neurodegenerative disorder may be, for example, Parkinson's disease, Alzheimer's disease or Huntington's disease. For each disease or disorder, the mRNA of the D<sub>3</sub> dopamine receptor, and/or of the  $\alpha 7$  AChR, and of a control gene are measured in PBLs of tested individuals suffering from said

25 disorder, and in PBLs of healthy control individuals, the ratio between the D<sub>3</sub> dopamine receptor mRNA and the control gene mRNA, and/or the ratio between  $\alpha 7$  AChR mRNA and the control gene mRNA for each individual is calculated, and the correlation between said increase or decrease is evaluated for each disorder or disease in the same

30 way as described herein in detail for schizophrenia.

In one embodiment, the invention relates to a method for the diagnosis and follow up of schizophrenia in an individual, comprising:

(i) measuring mRNA of  $D_3$  dopamine receptor and/or of  $\alpha 7$  AChR and of a control gene in PBLs of said individual and of at least one healthy control individual;

(ii) calculating the ratio between the  $D_3$  dopamine receptor mRNA and the control gene mRNA and/or the ratio between  $\alpha 7$  AChR mRNA and the control gene mRNA for  
5 each individual; and

(iii) evaluating the ratio between the ratios obtained in (ii) for the individual tested for schizophrenia and for the at least one healthy control individual, wherein an increase of above 1.6 fold, preferably 2-4, in the  $D_3$  dopamine receptor mRNA and/or a decrease of more than 20%, preferably 20-98%, in the  $\alpha 7$  AChR mRNA in the tested individual in  
10 comparison to healthy individuals, indicate that said tested individual has a high likelihood of having schizophrenia.

In order to carry out this assay, blood is obtained from individuals, PBLs are isolated therefrom, and total RNA is isolated from the lymphocytes by standard methods as well known in the art. The mRNA of the total RNA is then reverse-transcribed into  
15 cDNA that is used for PCR amplification using primers for the  $D_3$  dopamine receptor, for the  $\alpha 7$  AChR, and for a control house keeping gene such as  $\beta$ -actin,  $\alpha$ -actin, NADH or tubulin. Measuring the  $D_4$  dopamine receptor-mRNA can also serve as a control. Quantification of the PCR products by densitometry, PCR-ELISA, fluorescence techniques, or Southern blot, correlates to the mRNA levels of the  $D_3$  dopamine  
20 receptor,  $\alpha 7$  AChR, and of the control gene in the PBLs. For example, when the quantification of the PCR products is carried out by densitometry, the program, in a defined area, gives a number corresponding to the brightness intensity.

In one embodiment, the mRNA level of the  $D_3$  dopamine receptor, and/or of the  $\alpha 7$  AChR, and of a control gene of a tested individual, e.g. a schizophrenic individual, is  
25 compared with the mRNA level of the  $D_3$  dopamine receptor, and/or of the  $\alpha 7$  AChR, and of a control gene of a sole healthy individual, preferably of the same age and sex. In another embodiment, the comparison is made with a pool of PBLs of two or more healthy individuals.

In another aspect, the invention relates to a kit for use in the method of the  
30 invention. The kit comprises, for example, (i) means for isolating mRNA from PBLs; (ii) means for reverse transcription and for PCR; and (iii) means for detection of PCR products. The kit may also contain means for separating PBL from whole blood.

In one embodiment, the assay may be carried out by the use of DNA arrays or differential display.

#### **Brief Description of the Figures**

5 Figs. 1a-1c shows ethidium bromide staining of D<sub>3</sub>, D<sub>4</sub>, and  $\beta$ -actin PCR products obtained from mRNA of peripheral blood lymphocytes (PBLs) of schizophrenic (S) and control healthy (C) individuals.

Fig. 2 shows ethidium bromide staining of  $\beta$ -actin and  $\alpha 7$  AChR PCR products obtained from mRNA of PBLs of schizophrenic (Sp) and control healthy (Hl)  
10 individuals.

Fig. 3 shows a comparison of  $\alpha 7$  AChR/ $\beta$ -actin mRNA ratios in PBLs of healthy controls (HL), schizophrenic patients (S) and unmedicated patients (tested in their first hospitalization, FH).

Fig. 4 shows a comparison of  $\alpha 7$  AChR/ $\beta$ -actin mRNA ratios in PBL of healthy  
15 smokers and non-smokers.

#### **Description of Preferred Embodiments**

The invention will now be illustrated by the following non-limiting examples.

#### **Experimental**

**Patients.** Schizophrenic patients were recruited from Tyrat Hacarmel and Beer Yaacov Mental Health Centers, Israel, after providing written informed consent for participation in the study. The study has been approved by the Institutional Review Board for human studies in these two mental health centers. All patients were formally diagnosed  
25 according to the Diagnostic and Statistical Manual of Mental disorder-IV criteria and evaluated by using standard rating scales by a senior psychiatrist. Healthy individuals' age and sex matched the patient group as much as possible.

**Lymphocyte isolation.** Blood (40-50 ml for D<sub>3</sub> dopamine receptor, or 20-30 ml for the  
30  $\alpha 7$  AChR) was drawn from the cubital vein into a heparinized plastic syringe, and then transferred into a sterile 50-ml plastic tube. Blood samples were diluted with an equal volume of phosphate-buffered saline (PBS), were placed onto Ficoll-Paque gradients, and then were centrifuged for 30 minutes at 400xg. The lymphocyte layer was collected,



and washed twice in PBS. The resulting pellet was immediately frozen at  $-80^{\circ}\text{C}$  until RNA preparation.

**Reverse Transcription - PCR analysis:** Total RNA was isolated from lymphocytes by the guanidinium-thiocyanate method, and the amount and quality of RNA were determined by spectrophotometry and gel electrophoresis (2% agarose for the  $D_3$  dopamine receptor, or 1.5% for the  $\alpha 7$  AChR; GibcoBRL). Two  $\mu\text{g}$  of total RNA were reverse transcribed into first-strand cDNA using poly-dT-priming and 20 units of Molony murine leukemia virus reverse transcriptase. Two  $\mu\text{l}$  cDNA product (80 ng RNA) was used for the PCR amplification at a final concentration of 1X PCR buffer (Perkin-Elmer), and 1 U of Taq DNA polymerase (Perkin-Elmer) in a 25  $\mu\text{l}$  final volume. PCR was carried out in a DNA thermocycler (Minicycler MJ research, MA) for 23 cycles ( $\beta$ -actin), 38 cycles ( $D_3$  and  $D_4$  dopamine receptors), and 39 cycles ( $\alpha 7$  AChR). Annealing temperatures for  $\beta$ -actin, for  $D_3$  and for  $D_4$  dopamine receptors was  $60^{\circ}\text{C}$ , while that for  $\alpha 7$  AChR was  $57^{\circ}\text{C}$ . The amplification was found to be linear between 30 and 40 cycles for  $D_3$  and  $D_4$  dopamine receptors, as well as for the  $\alpha 7$  AChR, and between 19 and 25 cycles for  $\beta$ -actin.

The PCR primers for  $D_3$ -,  $D_4$ -dopamine receptors, for  $\alpha 7$  AChR, and for  $\beta$ -actin were designed to include at least one intron, to eliminate amplification of genomic DNA. Their sequences were as follows:

$D_3$  dopamine receptor - GGAGACGGAAAAGGATCCTCACTCG (nt 655-680);  
TCAGCAAGACAGGATC TTGAGGAAGG (nt 1203-1177).

$D_4$  dopamine receptor - CGGGATCCCACCCAGACTCCACC (nt 964-988);  
CGGAATTCCGTTGCGGAACTCGGC (nt 1240-1216).

$\alpha 7$  AChR receptor-AAGTTTGGGTCCTGGTCTTACG (nt 571-592);  
GATCATGGTGCTGGCGAAGTA (nt 978-958 ).

$\beta$ -actin- TGAAGTGTGACGTGGACATCCG (nt 96-117);  
GCTGTCACCTTCACCGTT CCAG (nt 543-522).

Quantification of PCR products was performed by using a densitometer and a SCION IMAGE (Frederick, MD) analysis, and/or PCR-ELISA.

PCR-ELISA: PCR was performed as described above except for the use of digoxigenin-labeled dNTPs. PCR products were incubated with biotinylated specific internal primers of the tested fragments that were immobilized in streptavidin-coated microtiter plates. The biotinylated internal primers served as capture probes. The bound digoxigenin-labeled PCR-products were then incubated with anti-digoxigenin-peroxidase conjugate that bound to the digoxigenin residues in the labeled PCR products. Peroxidase substrate solution was added, and the color developed was measured in a microtiter-plate reader.

#### 10 Example 1

Table 1 summarizes the details (ages, sexes, and diagnoses) of schizophrenic patients and healthy controls from whom blood samples were obtained. RT-PCR was performed on total RNA preparations from these blood samples with primers specific for D<sub>3</sub> or D<sub>4</sub> dopamine receptor, and  $\beta$ -actin as a control. The specific PCR products were resolved on 2% agarose gels, and their sequences were verified. For each patient, a sex- and optimal age-matched healthy control was used, and the level of specific dopamine receptor mRNA was compared between sick and healthy patients. As depicted in Fig. 1 (a and b) for several representative patients, the signals for D<sub>3</sub> receptor mRNA were significantly higher in schizophrenic patients than in healthy controls. This increase was found to be specific for the D<sub>3</sub> dopamine receptor, because no significant differences in the intensities of D<sub>4</sub> receptor bands were detected between schizophrenic patients and healthy controls (Figs. 1b, 1c).

Quantification of the intensities of the specific D<sub>3</sub> dopamine receptor bands was performed by densitometry. The results obtained for 13 patients are summarized in Table 2. Each schizophrenic patient was compared with a sex- and optimal age-matched healthy individual. For each of them, a ratio of the measured density value for D<sub>3</sub> receptor to the value for  $\beta$ -actin was determined. The ratio of these two values for a patient and a matched healthy control, respectively, represents the increased level (in folds) in D<sub>3</sub> specific mRNA in a given patient. As shown in Table 2, the increased levels obtained for the 13 patients range between 1.59 and 7.45 (mostly between 2-3). This increase in D<sub>3</sub> receptor mRNA in schizophrenic patients is significantly higher than the reported increases in binding levels and other recently suggested peripheral markers for

schizophrenia (Avissar et al., 1997). Furthermore, the increase in D<sub>3</sub> receptor mRNA was not affected by different drug treatments. Although some of the patients received typical treatment and some atypical treatment (see Table 1), it can be noted that all patients exhibited a similar range of increase indicating that this was not a result of specific dopamine-receptor subtype blockade and up-regulation. Moreover, the present inventors found that this increase was not the consequence of a dopamine receptor antagonist treatment, because non-medicated patients (S12, S13) showed a similar increase in D<sub>3</sub> level (see Tables 1 and 2).

Another way to quantify the differences in a specific mRNA level was obtained from PCR-ELISA experiments (see Experimental part). Table 3 summarizes the results obtained from 6 patients. The increased mRNA levels observed are between 1.6 and 3.38 (average increase  $2.30 \pm 0.63$ ). It should be noted that there is a relatively good agreement between the quantitative values obtained by densitometry and by PCR-ELISA (see patients S1, S4 and S6 in Tables 2 and 3).

It should be added that the use of sex- and/or age-matched controls does not appear to be critical. The present inventors demonstrated that the differences in D<sub>3</sub> specific mRNA levels between schizophrenics and healthy individuals, determined by either densitometry or PCR-ELISA, were similar when compared with additional, not necessarily matched, controls (Table 4). This observation may be valuable in designing a practical assay wherein PBL from two or more healthy individuals may be pooled for use as a control.

In conclusion, these findings strongly suggest that D<sub>3</sub>-receptor mRNA levels in PBLs may serve as a convenient and reliable peripheral marker for schizophrenia, thus assist in early diagnosis (which is frequently unclear), and possible follow-up of the illness.

### Example 2

Thirty four patients were included in this study, 14 men and 20 women, ranging from 18 to 67 year of age. Of these, 20 were hospitalized schizophrenic patients, and 14 unmedicated patients that were examined during their first hospitalization. 21 healthy controls were studied, 11 nonsmokers and 10 smokers, 8 male and 13 female ranging from 31 to 62 years of age. Table 5 summarizes the details (age, sex and diagnosis) of schizophrenic patients and healthy controls participating in this study.

RNA was prepared from blood samples and RT-PCR was performed on total RNA, using specific primers for the  $\alpha 7$  AChR, and for  $\beta$ -actin as a control. The specific PCR products were resolved on 1.5% agarose gels. As depicted in Fig. 2, the signals for  $\alpha 7$  AChR were significantly lower in 3 schizophrenic patients than in 3 healthy controls.

5 Quantification of the intensities of the specific  $\alpha 7$  AChR and  $\beta$ -actin bands was performed by densitometry. The results obtained in 28 experiments are summarized in Table 6a. For each individual, a ratio of the measured density value for the  $\alpha 7$  AChR to the value for  $\beta$ -actin was determined. Each patient was tested 1-4 times. As seen in Table 6a, 10 of the 34 tested patients had no detectable band for  $\alpha 7$  AChR. The  $\alpha 7$

10 AChR/ $\beta$ -actin ratios for these patients was arbitrarily determined as  $<0.1$  (lower than the smallest calculated ratio in Table 6a). The  $\alpha 7$  AChR/ $\beta$ -actin ratios for healthy controls (HL), schizophrenic patients (S) and unmedicated patients (first hospitalization, FH) obtained in all experiments are depicted in Figure 3. The average values were  $0.88 \pm 0.18$ ,  $0.36 \pm 0.30$  and  $0.34 \pm 0.26$  for healthy controls, schizophrenic patients and

15 unmedicated patients, respectively.

To determine the significance of the difference between healthy and schizophrenic patients, the  $\alpha 7$  AChR/ $\beta$ -actin ratios obtained for 11 different healthy controls and for 14 different schizophrenic patients were compared by Sign test. In each experiment, the values obtained for the schizophrenic patients are significantly lower

20 than for the corresponding healthy individuals ( $P < 0.004$ ).

The reduction in the level of  $\alpha 7$  AChR mRNA observed in schizophrenic patients was calculated by the following equation:  $100 - 100[(\alpha 7 \text{ AChR}/\beta\text{-actin S}) / (\alpha 7 \text{ AChR}/\beta\text{-actin Hav})]$ . First, the average  $\alpha 7$  AChR/ $\beta$ -actin ratios of all healthy controls in a given experiment was calculated (Hav). The decrease (%) of the  $\alpha 7$  AChR mRNA for

25 each patient was obtained after subtracting the % of  $(\alpha 7 \text{ AChR}/\beta\text{-actin S}) / (\alpha 7 \text{ AChR}/\beta\text{-actin Hav})$  ratio from 100%. The % of decrease for all experiments were calculated, and are depicted in the last column of Table 6a. As seen in this Table, there were only 8 determinations (representing 6 different patients), in which the percent decrease was lower than 20%. All the other determinations in patients resulted in

30 significant decreases in the  $\alpha 7$  AChR mRNA levels, ranging from 20% to 98% decrease. So far, the present inventors have not observed a correlation between the percent decrease of  $\alpha 7$  AChR mRNA and the disease state. However, it is interesting to point

out that one unmedicated patient (FH2) that was tested in his first hospitalization, and exhibited a very low % decrease in its  $\alpha 7$  AChR mRNA (5.1%, representing an average of three independent determinations), turned to be non schizophrenic following detailed psychiatric evaluation.

5           The incidence of smoking in a mental illness, particularly in schizophrenia, is much higher than in the general population, 74-92% compared to 30-55%, respectively (Olinicy et al., 1999). We have, therefore, tested whether smoking by itself has an effect on  $\alpha 7$  AChR mRNA levels. Blood samples from healthy smokers that smoke a pack of cigarettes a day, and from healthy nonsmokers were analyzed for their  $\alpha 7$  AChR mRNA  
10   levels. As depicted in Table 6b, there were no significant differences in the  $\alpha 7$  AChR/ $\beta$ -actin ratios of smokers and nonsmokers. This suggests that the decrease in  $\alpha 7$  AChR mRNA levels in schizophrenic patients is not a result of smoking. The  $\alpha 7$  AChR/ $\beta$ -actin ratios for healthy smokers and nonsmokers are depicted in Figure 4, demonstrating average ratios of  $0.83 \pm 0.097$  and  $0.89 \pm 0.097$ , for smokers and nonsmokers,  
15   respectively.

          The dopaminergic hypothesis of schizophrenia proposes that hyperactivity of dopamine transmission is responsible for the symptoms of this disorder. In the first example of the present invention we have demonstrated increased levels of D3 dopamine receptor mRNA in PBLs of schizophrenic patients, when compared with the levels in  
20   healthy controls. In this example, the present inventors analyzed the mRNA levels of both D3 dopamine receptor and of the  $\alpha 7$  AChR in PBLs of a small group of patients. As expected, the levels of D3 dopamine receptor mRNA were increased, whereas the levels of  $\alpha 7$  AChR mRNA were decreased. As shown in Table 7, an increase of 55.52% in the levels of D3 receptor mRNA and a decrease of 63.66% in the levels of  $\alpha 7$  AChR  
25   mRNA were observed for patient SC8. The availability of two different biological markers (the mRNA level of D3 dopamine receptor and  $\alpha 7$  AChR) that can be both tested in PBLs makes the evaluation of schizophrenic patients by a peripheral and objective test, rather promising. Moreover, the fact that the mRNA level of these two receptor mRNAs changes in an opposite direction in schizophrenia, i.e., the gene  
30   expression of D3 receptor increases, whereas the gene expression of  $\alpha 7$  AChR decreases in PBLs of schizophrenic patients, that correlates with the changes in these receptors in

the brain (as observed in post mortem schizophrenic patients), makes these assays experimentally convenient and reliable.

In conclusion, the decreased levels of mRNA of the  $\alpha 7$  AChR in PBLs of schizophrenic patients, as presented herein, is consistent with earlier reports  
5 demonstrating a decrease in  $\alpha 7$  AChR in post mortem brains of schizophrenic patients. Such correlation between decreased levels of  $\alpha 7$  AChR mRNA in PBLs and the expression of schizophrenia justifies its application as a biological marker for this disease.

10

**Table 1. Characterization of patients (Example 1).****Schizophrenic Patients:**

Number	Age	Sex	Diagnosis	Comments
S1	21	M	Chronic negative schizophrenia	
S2	27	M	Chronic negative schizophrenia	
S3	25	M	Chronic negative schizophrenia	
S4	27	F	Positive psychosis	
S5	49	F	Acute schizophrenia	
S6	57	F	Residual Schizophrenia	
S7	41	M	Undifferentiated schizophrenia	
S8	54	M	Paranoid schizophrenia	
S9	47	M	Undifferentiated schizophrenia	
S10	42	M	Undifferentiated schizophrenia	
S11	42	M	Paranoid schizophrenia	
S12	21	M	Undifferentiated schizophrenia	Non-medicated
S13	40	F	Paranoid schizophrenia	Non-medicated

**Healthy Controls:**

Number	Age	Sex
C1	45	F
C2	37	F
C3	37	M
C4	62	F
C5	22	M
C6	44	M
C7	31	M
C8	32	M
C9	49	F
C10	27	M
C11	36	F

**Table 2. Densitometric evaluation of D<sub>3</sub> and D<sub>4</sub> mRNA levels in patients as compared with their levels in healthy individuals. (Example 1)**

**A.**

Schizophrenic patients:

Controls:

Number	$\beta$ -actin Arb. units	D <sub>3</sub> Arb. units	D <sub>3</sub> / $\beta$ -actin	Number	$\beta$ -actin Arb. units	D <sub>3</sub> Arb. units	D <sub>3</sub> / $\beta$ -actin	D <sub>3</sub> fold increase (S/C)
S1	67	51	0.671	C3	86	23	0.267	2.513
S2	87	56	0.643	C3	86	23	0.267	2.408
S3	82	60	0.731	C3	86	23	0.267	2.737
S4	98	100	1.02	C2	95	61	0.642	1.588
S5	55	143	2.600	C2	58	59	1.017	2.556
S6	75	153	2.040	C4	79	70	0.886	2.302
S7	19	171	9.000	C10	85	163	1.917	4.694
S8	138	426	3.087	C8	425	176	0.414	7.456
S9	89	60	0.674	C7	121	28	0.231	2.917
S10	107	71	0.663	C7	121	28	0.231	2.870
S11	303	277	0.914	C3	271	29	0.107	6.644
S12	319	227	0.711	C7	273	86	0.315	2.257
S13	237	354	1.493	C9	199	130	0.653	2.870

**B.**

Schizophrenic patients:

Controls:

Number	$\beta$ -actin Arb. units	D <sub>4</sub> Arb. units	D <sub>4</sub> / $\beta$ -actin	Number	$\beta$ -actin Arb. units	D <sub>4</sub> Arb. units	D <sub>4</sub> / $\beta$ -actin	D <sub>4</sub> fold increase (S/C)
S1	74	70	0.945	C3	67	70	1.044	0.905
S2	71	72	1.010	C3	67	70	1.044	0.967
S3	72	74	1.027	C3	67	70	1.044	0.983
S4	67	71	1.059	C2	82	73	0.890	1.189



**Table 3. Evaluation by PCR-ELISA of D3 mRNA levels in patients as compared with their levels in healthy individuals. (Example 1)**

Schizophrenic patients:

Controls:

Number	$\beta$ -actin (O.D)	D <sub>3</sub> (O.D)	D <sub>3</sub> / $\beta$ -actin	Number	$\beta$ -actin (O.D)	D <sub>3</sub> (O.D)	D <sub>3</sub> / $\beta$ -actin	D <sub>3</sub> fold increase (S/C)
S1	0.556	0.868	1.56	C8	0.918	0.552	0.601	2.595
S2	0.808	2.225	2.75	C9	0.405	0.33	0.814	3.378
S3	0.224	0.253	1.13	C8	0.533	0.272	0.510	2.215
S4	0.629	0.394	0.626	C8	0.876	0.316	0.360	1.738
S5	0.340	0.823	2.420	C2	0.365	0.533	1.46	1.657
S6	0.339	0.899	2.652	C3	0.368	0.444	1.206	2.199

**Table 4. Evaluation of D3 mRNA levels in patients as compared with their levels in several healthy individuals (Example 1)**

Schizophrenia patients: Controls:

Number		Number	D <sub>3</sub> / $\beta$ -actin	Ratio
S8	3.087	C8	0.414	7.456
		C9	0.498	6.198
S5	2.600	C2	1.017	2.6
		C3	1.145	2.27
		C4	0.886	2.934
S6	2.040	C2	1.017	2.005
		C3	1.145	1.78
		C4	0.886	2.302
S4	1.02	C2	0.642	1.588
		C8	0.656	1.554

**Table 5: Characteristics of patients and healthy donors (Example 2)**

Sample	Age	Gender	Diagnosis
<b>Healthy controls (nonsmokers)</b>			
HL16	49	F	
HL15	32	M	
HL14	31	M	
HL10	37	M	
HL5	40	M	
HL9	39	F	
HL18	36	F	
HL11	62	F	
HL6	35	M	
HL 19	32	M	
HL 20	35	F	
<b>Healthy smokers</b>			
SM1	52	F	
SM2	35	M	
SM3	35	F	
SM4	41	M	
SM6	44	F	
SM7	37	F	
SM8	45	F	
SM9	54	F	
SM10	55	F	
SM11	50	F	
<b>Schizophrenic patients</b>			
SP1	21	M	Schizophrenia-residual type
SP2	27	M	Schizophrenia-paranoid type
SP3	25	M	Schizophrenia-undifferentiated type
SP7	49	F	Schizophrenia-paranoid type
SP8	57	F	Schizophrenia-residual type
SP9	31	F	Schizophrenia-paranoid type
SP12	56	F	Schizophrenia-undifferentiated type
SP13	54	M	Schizophrenia-paranoid type
SP15	31	F	Schizophrenia-paranoid type
SP19	54	F	Schizophrenia-paranoid type
SP20	45	F	Schizophrenia
BY8	39	M	Schizophrenia-residual type
BY9	62	F	Disorganized Schizophrenia
BY10	67	F	Disorganized Schizophrenia
BY11	50	M	Schizophrenia
SC6	51	M	Schizophrenia
SC7	24	F	Schizophrenia
SC8	64	F	Schizophrenia
SC10	63	F	Schizophrenia
SP5	65	F	Schizophrenia-undifferentiated type

Table 5 (continued)

Schizophrenic patients (First hospitalization)			
BY14	40	F	Schizophrenia-paranoid type
FH2	30	F	
FH3	27	M	Personality disorder
FH4	40	M	Psychotic episode
FH5	18	M	Schizophrenia
FH6	21	M	Personality disorder
FH7	26	M	Acute psychotic disorder
FH9	26	F	Schizophrenia-moderately ill
FH10	23	F	Schizophrenia-markedly ill
FH11	20	F	Schizophrenia-moderately ill
FH12	35	M	Acute psychotic disorder
FH13	38	M	Schizophrenia-moderately ill
FH14	20	F	Acute psychotic disorder
FH15	48	F	Acute psychotic disorder

Table 6a: Evaluation of  $\alpha 7/\beta$ -actin mRNA levels of schizophrenic patients compared to healthy controls (Example 2)

Exp. No.	Schizophrenic patients					Healthy controls				
	Sample No.	$\beta$ -actin (arb. Units)	$\alpha 7$ (arb. Units)	$\alpha 7/\beta$ -actin		Sample No.	$\beta$ -actin (arb. Units)	$\alpha 7$ (arb. Units)	$\alpha 7/\beta$ -actin	Decrease %
1	SP1	206.49	75.77	.37		HL16	228.25	223.65	.98	59.99
	SP2	220.25	109.33	.496		HL15	235.74	230.62	.99	45.87
	SP3	215.3	91.46	.42		HL14	206.45	163.76	.79	53.68
2	SP7	155.53	134.14	.86		HL16	166.3	152.18	.92	17.35
	SP8	168.35	140.64	.84		HL5	131.47	154.08	1.17	19.95
	SP9	135.95	112.06	.82						21.01
3	SP3	133.48	148.31	.89		HL16	104.04	138.75	1.33	23.08
	SP12	143.23	123.66	.86		HL15	130.7	118.01	.90	25.07
						HL14	106.07	129.44	1.22	
4	SP19	173.83	93.35	.54		HL14	175.14	138.94	.79	41.15
	SP15	159.95	undetectable	<0.1		HL19	175.91	164.57	.94	>89.05
						HL16	164.39	165.8	1.01	
5	BY8	108.54	80.63	.74		HL16	114.12	131.7	1.15	35.63
	BY10	97.75	undetectable	<0.1						>91.31
	BY11	101.5	undetectable	<0.1						>91.31
6	BY14	182.8	114	.62		HL18	181.07	128.85	.69	10.27
	FH2	221.62	188.4	.85		HL18	241.88	210.59	.87	3.97
	SP19	228.01	138.64	.61		HL15	236.86	194.03	.82	31.31
7	SP20	171.5	undetectable	<0.1		HL11	235.08	227.08	.97	>88.72
	SP3	202.13	undetectable	<0.1						>88.72
	BY10	240.7	179.56	.75						15.73
8	SC7	170.88	144.4	.85		HL6	148.91	144.1	.97	12.68
	SC10	124.7	95.67	.77						20.72
	FH6	162.22	undetectable	<0.1						>90.30
	SC6	158.41	undetectable	<0.1						>90.30

Table 6 a (continued)

9	SC7	201.25	127.85	.64	HL6	187.14	157.69	.84	24.61
	SC10	215.77	86.5	.40					52.42
	FH6	201.53	81.66	.41					51.91
	FH7	165.53	68.99	.42					50.54
	SC6	180.86	undetectable	<0.1					>88.10
	SC8	209.27	undetectable	<0.1					>88.10
10	FH2	227.06	199.01	.88	HL11	229.63	170.67	0.74	-9.80
					HL14	239.21	204.11	0.85	
11	SP3	190.77	51.58	.27	HL9	190.34	123.79	.65	58.43
12	SP1	166.62	90.67	.54	HL5	155.5	100.14	.64	41.36
	SP2	167.84	90	.54	HL9	164.2	103.37	.63	42.22
	SP3	157.41	undetectable	<0.1	HL9	167.36	102.55	.61	>85.62
	SP20	145.1	undetectable	<0.1	HL14	167.28	150.2	.90	>85.62
13	SP15	126.61	undetectable	<0.1	HL10	154.99	85.57	0.55	>81.31
					HL9	145.48	74.96	0.52	
14	SP3	186.89	undetectable	<0.1	HL9	177.55	137.62	0.78	>86.85
	SP15	166.82	undetectable	<0.1	HL10	170.43	139.54	0.82	>86.85
	SP19	181.28	undetectable	<0.1	HL15	170.05	100.22	0.59	>86.85
15	SP9	166.45	undetectable	<0.1	HL15	168.02	90.39	0.54	>80.59
	SP19	139.61	undetectable	<0.1	HL16	168.81	91.01	0.54	>80.59
					HL10	166.02	81.96	0.49	
					HL18	168.06	82.67	0.49	
16	SP19	220.14	undetectable	<0.1	HL14	217.55	131.95	0.61	>85.30
	SP20	197.13	undetectable	<0.1	HL5	203.7	153.4	0.75	>85.30
17	SP15	179.26	undetectable	<0.1	HL9	209.93	146.15	0.70	>85.72
18	FH2	61.84	26.08	0.52	HL18	76.29	46.87	0.61	21.94
	FH3	54.38	4.93	0.11	HL11	72.83	52.98	0.73	83.00

Table 6 a (continued)

19	FH3 SP9 SP5 SP12 SP13 FH4	63.37 52.77 32.03 45.16 20.1 60.35	6.54 undetectable undetectable undetectable undetectable undetectable	0.10 <0.1 <0.1 <0.1 <0.1 <0.1	HL5	29.82	14.75	0.49	74.90 >79.60 >79.60 >79.60 >79.60 >79.60
20	FH7 FH9 FH5	43.61 99.78 81.75	10 14.48 undetectable	0.23 0.15 <0.1	SM7 SM8 SM9 SM10	95.28 105.05 84.78 89	55.89 72.68 49.92 68.77	0.59 0.69 0.59 0.77	65.26 78.01 >84.85
21	FH9 FH7 SC6	92.99 94.76 79.03	50.54 undetectable undetectable	0.54 <0.1 <0.1	SM9	81.75	69.1	0.85	35.70 >88.24 >88.24
22	FH9 FH7	63.85 53.35	32.77 undetectable	0.51 <0.1	SM9	63.48	50.93	0.80	36.03 >87.5
23	SC8 SC6	66.84 39.87	6.25 undetectable	0.09 <0.1	C3	84.21	17.32	0.21	54.54 >52.39
24	FH9 FH10	92.3 66.85	47.22 undetectable	0.51 <0.1	H2 HL6	127.12 127.53	111.29 133.84	0.88 1.05	46.85 >89.64
25	FH11 FH12 FH13 FH14 FH15	95.22 101.4 93.01 72.02 101.95	76.43 92.47 82.36 34.75 26.55	0.80 0.91 0.89 0.48 0.26	C3	99.08	103.75	1.05	23.35 12.91 15.44 53.92 75.13
26	FH15 FH14	108.85 95.75	38.52 undetectable	0.35 <0.1	C3	120.6	119.12	0.99	64.17 >89.9
27	SC8 FH14 FH15	67.04 47.29 74.17	0.75 3.21 29.25	0.01 0.07 0.39	C3	71.26	64.85	0.91	98.78 92.54 56.67
28	BY9	212.5	94.95	0.45	HL18	187.8	114.39	0.61	35.71

**Table 6b: Evaluation of  $\alpha 7/\beta$  -actin mRNA levels of smokers compared to non smokers (Example 2)**

Exp No.	Smokers (healthy controls)				Non smokers (healthy controls)				Decrease %
	Sample No.	$\beta$ -actin (arb. Units)	$\alpha 7$ (arb. Units)	$\alpha 7/\beta$ -actin	Sample No.	$\beta$ -actin (arb. Units)	$\alpha 7$ (arb. Units)	$\alpha 7/\beta$ -actin	
1	SM1	245.51	228.35	.93	HL11	229.63	170.67	0.74	-16.52
	SM3	235.09	187.48	.80	HL14	239.21	204.11	0.85	.26
	SM4	228.96	192.34	.84					-5.24
	SM6	148.88	125.74	.84	HL6	148.91	144.1	.97	12.72
2	SM7	170.86	125.14	.73					24.31
	SM8	170.29	149.14	.88					9.50
	SM7	216.7	223.81	1.03	HL6	187.14	157.69	.84	-22.57
	SM8	165.87	117.62	.71					15.85
4	SM1	240.84	202.56	0.84	HL18	241.88	210.59	.87	4.99
					HL15	236.86	194.03	.82	
					HL11	235.08	227.08	.97	

**Table 7: Comparison of  $\alpha 7$  and  $D_3$  mRNA levels**

Sample	$\beta$ -actin (arb. Units)	$\alpha$ 7 (arb. Units)	$\alpha$ 7/ $\beta$ -actin	Decrease		$\beta$ -actin (arb. Units)	D3 (arb. Units)	D3/ $\beta$ -actin	Increase	
				%	Fold				%	Fold
C3	83.36	67.28	0.81			83.36	0.65	0.007		
SC8	67.31	19.86	0.30	63.66	2.7	67.31	1.18	0.02	55.52	2.86

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## CLAIMS:

1. A method for the diagnosis and follow up of a mental disorder or of a neurodegenerative disorder in an individual, comprising:
  - 5 (i) measuring mRNA of  $D_3$  dopamine receptor and/or of  $\alpha 7$  nicotinic acetylcholine receptor ( $\alpha 7$  AChR) and of a control gene in peripheral blood lymphocytes (PBLs) of said individual and of at least one healthy control individual;
  - (ii) calculating the ratio between the  $D_3$  dopamine receptor mRNA and the control gene mRNA and/or the ratio between  $\alpha 7$  AChR mRNA and the control gene mRNA for  
10 each individual; and
  - (iii) evaluating the ratio between the ratios obtained in (ii) for the tested individual and for the at least one healthy control individual, wherein an increase in the  $D_3$  dopamine receptor mRNA and/or a decrease in the  $\alpha 7$  AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood  
15 of having said mental disorder or neurodegenerative disorder, wherein said increase in the  $D_3$  dopamine receptor mRNA and/or decrease in the  $\alpha 7$  AChR mRNA in the tested individual is correlated to said mental disorder or neurodegenerative disorder.
2. A method according to claim 1 wherein said mental disorder is schizophrenia, manic  
20 depression, Tourette syndrom, or a similar disorder.
3. A method according to claim 1 wherein said neurodegenerative disorder is Parkinson's disease, Alzheimer's disease, or Huntington disease.
- 25 4. A method according to claim 1 or 2 for the diagnosis and follow up of schizophrenia in an individual, comprising:
  - (i) measuring mRNA of  $D_3$  dopamine receptor and/or of  $\alpha 7$  AChR and of a control gene in PBLs of said individual and of at least one healthy control individual;
  - (ii) calculating the ratio between the  $D_3$  dopamine receptor mRNA and the control  
30 gene mRNA and/or the ratio between the  $\alpha 7$  AChR and the control gene mRNA for each individual; and
  - (iii) evaluating the ratio between the ratios obtained in (ii) for the individual tested for schizophrenia and for the at least one healthy control individual, wherein an increase of

above 1.6 fold in the D<sub>3</sub> dopamine receptor mRNA and/or a decrease of more than 20% in the  $\alpha$ 7 AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood of having schizophrenia.

- 5     5. A method according to claim 4 for the diagnosis and follow up of schizophrenia in an individual, comprising:
- (i)     measuring mRNA of D<sub>3</sub> dopamine receptor and of a control gene in PBLs of said individual and of at least one healthy control individual;
- (ii)     calculating the ratio between the D<sub>3</sub> dopamine receptor mRNA and the control  
10     gene mRNA for each individual; and
- (iii)     evaluating the ratio between the ratios obtained in (ii) for the individual tested for schizophrenia and for the at least one healthy control individual, wherein an increase of above 1.6 fold in the D<sub>3</sub> dopamine receptor mRNA in the tested individual in comparison to healthy individuals, indicates that said tested individual has a high likelihood of having  
15     schizophrenia.
6. A method according to claim 4 for the diagnosis and follow up of schizophrenia in an individual, comprising:
- (i)     measuring mRNA of the  $\alpha$ 7 AChR and of a control gene in PBLs of said  
20     individual and of at least one healthy control individual;
- (ii)     calculating the ratio between the  $\alpha$ 7 AChR mRNA and the control gene mRNA for each individual; and
- (iii)     evaluating the ratio between the ratios obtained in (ii) for the individual tested for schizophrenia and for the at least one healthy control individual, wherein a decrease of  
25     more than 20% in the  $\alpha$ 7 AChR mRNA in the tested individual in comparison to healthy individuals, indicates that said tested individual has a high likelihood of having schizophrenia.
7. A method according to any one of Claims 1 to 6, wherein the mRNA of D<sub>3</sub> dopamine  
30     receptor, of  $\alpha$ 7 AChR and of the control gene in step (i) is measured by reverse transcription-polymerase chain reaction (RT-PCR).
8. A method according to any one of Claims 1 to 7, wherein the control gene is  $\beta$ -actin.

9. A method according to any one of Claims 1 to 8, which comprises calculating in step  
(ii) the ratio between the D<sub>3</sub> dopamine receptor mRNA and/or the ratio between the  $\alpha$ 7  
AChR and the control gene mRNA of the individual tested for a mental or  
5 neurodegenerative disorder and the ratio between the D<sub>3</sub> dopamine receptor mRNA and/or  
the ratio between the  $\alpha$ 7 AChR and the control gene mRNA of a sole healthy individual.
10. A method according to any one of Claims 1 to 9, wherein said mental disorder is  
schizophrenia and in the schizophrenic individual the increase of D<sub>3</sub> dopamine receptor  
10 mRNA evaluated in step (iii) is of 2-4 folds and/or the decrease of the  $\alpha$ 7 AChR mRNA  
evaluated in step (iii) is 20 - 98%, in comparison to healthy individuals,.
11. A kit for use in a method according to any one of claims 1 to 10.
- 15 12. A kit according to claim 11, comprising:
- (i) means for isolating mRNA from PBL;
  - (ii) means for reverse transcription and for PCR; and
  - (iii) means for detection of PCR products.
- 20 13. A kit according to claim 12, further comprising means for separating PBL from whole  
blood.

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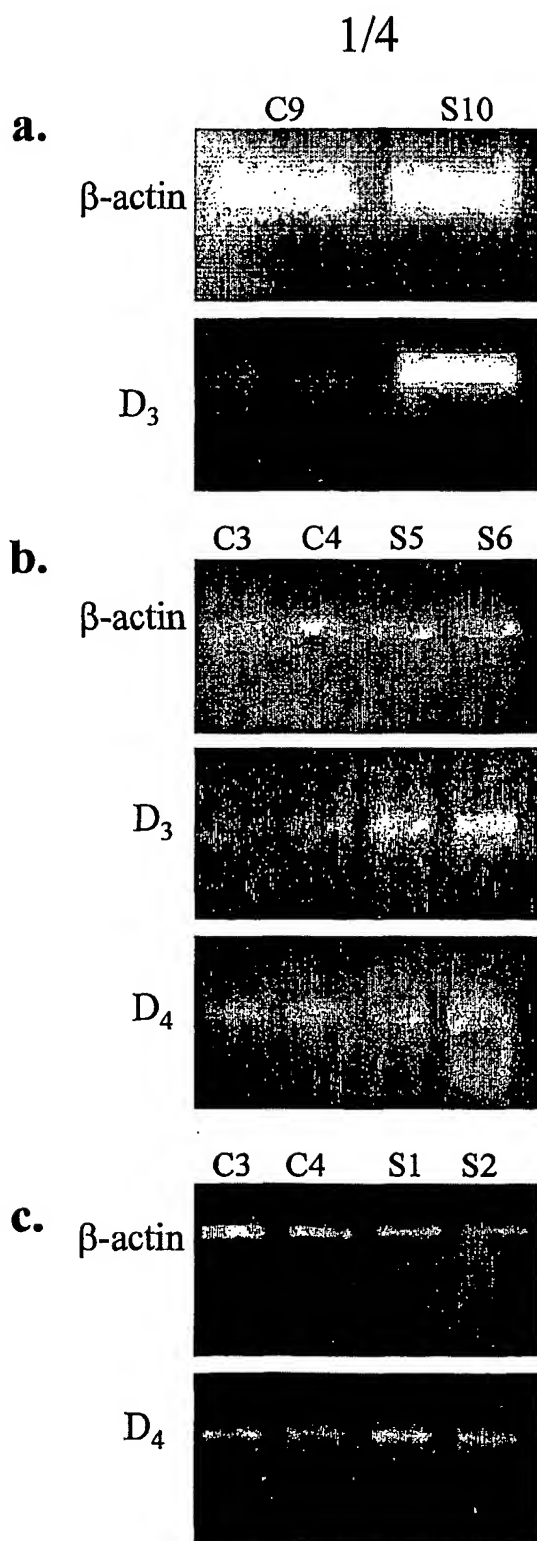


Fig. 1

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HL16, HL15, HL14, SP1, SP2, SP3

 $\beta$ -actin $\alpha$ 7 AChR

Fig. 2

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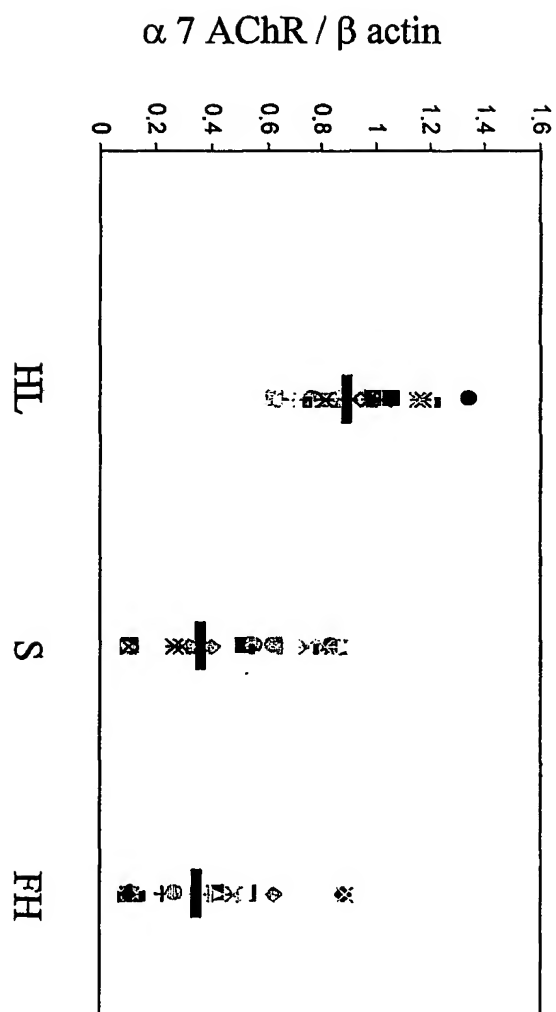


Fig. 3



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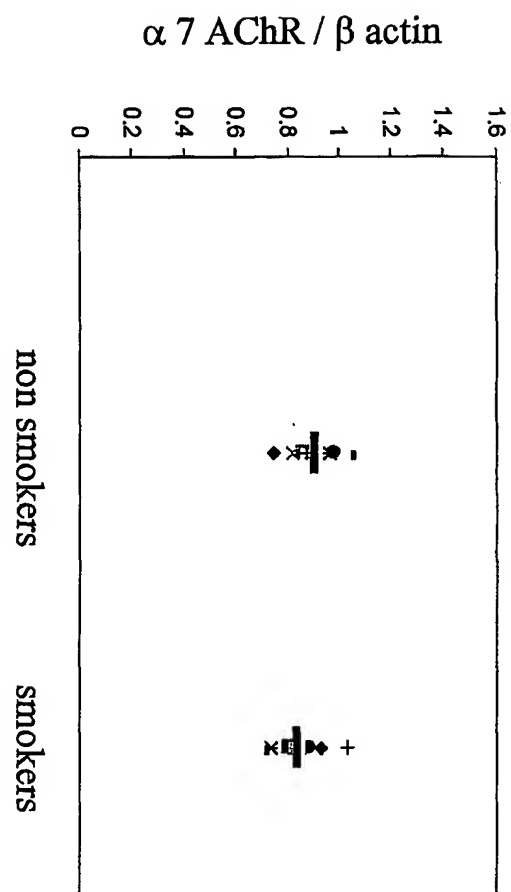


Fig. 4

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- (71) Applicant (for all designated States except US): YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE DIAGNOSIS AND FOLLOW UP OF SCHIZOPHRENIA AND OTHER MENTAL AND NEURODEGENERATIVE DISORDERS

(57) Abstract: A method for the diagnosis and follow up of a mental disorder or of a neurodegenerative disorder in an individual, comprises: (i) measuring mRNA of D<sub>3</sub> dopamine receptor and/or of α7 nicotinic acetylcholine receptor (α7 AChR) and of a control gene in peripheral blood lymphocytes (PBLs) of said individual and of at least one healthy control individual; (ii) calculating the ratio between the D<sub>3</sub> dopamine receptor mRNA and the control gene mRNA and/or the ratio between α7 AChR mRNA and the control gene mRNA for each individual; and (iii) evaluating the ratio between the ratios obtained in (ii) for the tested individual and for the at least one healthy control individual, wherein an increase in the D<sub>3</sub> dopamine receptor mRNA and/or a decrease in the α7 AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood of having said mental disorder or neurodegenerative disorder, wherein said increase in the D<sub>3</sub> dopamine receptor mRNA and/or decrease in the α7 AChR mRNA in the tested individual is correlated to said mental disorder or neurodegenerative disorder. When the mental disorder is schizophrenia, an increase of above 1.6 fold in the D<sub>3</sub> dopamine receptor mRNA and/or a decrease of more than 20% in the α7 AChR mRNA in the tested individual in comparison to healthy individuals, indicate that said tested individual has a high likelihood of having schizophrenia.

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 01/00761

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, EPO-Internal, WPI Data, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAGAI Y ET AL: "Decrease of the D3 dopamine receptor mRNA expression in lymphocytes from patients with Parkinson's disease." NEUROLOGY, vol. 46, no. 3, 1996, pages 791-795, XP009003027 ISSN: 0028-3878 cited in the application abstract page 791, right-hand column -page 792, left-hand column figure 3A  --- -/--	1-10



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*P\* document published prior to the international filing date but later than the priority date claimed

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\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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\*Z\* document member of the same patent family

Date of the actual completion of the international search

13 January 2003

Date of mailing of the international search report

30/01/2003

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 01/00761

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HELLSTÖM-LINDAHL ET AL.: "Expression of nicotinic receptor subunit mRNAs in lymphocytes from normal and patients with Alzheimer's disease." ALZHEIMER'S RESEARCH, vol. 3, no. 1-2, 1997, pages 29-36, XP009002917 cited in the application abstract page 30, left-hand column, line 2, paragraph 2 -page 32, left-hand column, paragraph 1 page 33, left-hand column, paragraph 2 -----	1-10
P,X	ILANI TAL ET AL: "A peripheral marker for schizophrenia: Increased levels of D3 dopamine receptor mRNA in blood lymphocytes." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 98, no. 2, 16 January 2001 (2001-01-16), pages 625-628, XP002225754 January 16, 2001 ISSN: 0027-8424 the whole document -----	1-10
P,X	KWAK ET AL.: "Change of dopamine receptor mRNA expression in lymphocyte of schizophrenic patients." BMC MEDICAL GENETICS, 'Online! vol. 2, 5 March 2001 (2001-03-05), pages 1-9, XP002225755 Retrieved from the Internet: <URL:http://www.biomedcentral.com/1471-235 0/2/3> 'retrieved on 2002-12-19! abstract page 5, left-hand column, paragraph 2 -right-hand column, paragraph 1 figure 4 -----	1-10

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IL 01/00761

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 11-13  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 11-13

Present claims 11-13 relate to a kit defined by reference to desirable characteristics or properties, namely

- (i) suitable for use in a method according to any one of claim 1 to 10
- (ii) suitable for isolating mRNA from PBL
- (iii) suitable for reverse transcription and for PCR
- (iv) suitable for detection of PCR products
- (v) suitable for separating PBL

The claims cover all kits having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only any such kit. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the kit by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out with respect to claims 11-13.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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